

Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer

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Abstract

Several lines of evidence indicate that during transformation epithelial cancer cells can acquire mesenchymal features via a process called epithelial-to-mesenchymal transition (EMT). This process endows cancer cells with increased invasive and migratory capacity, enabling tumour dissemination and metastasis. EMT is associated with a complex metabolic reprogramming, orchestrated by EMT transcription factors, which support the energy requirements of increased motility and growth in harsh environmental conditions. The discovery that mutations in metabolic genes such as *FH*, *SDH* and *IDH* activate EMT provided further evidence that EMT and metabolism are intertwined. In this review, we discuss the role of EMT in cancer and the underpinning metabolic reprogramming. We also put forward the hypothesis that, by altering chromatin structure and function, metabolic pathways engaged by EMT are necessary for its full activation.

Background

In the last decades, cancer research uncovered the many enabling features of tumours cells [1]. Among these, activation of epithelial-to-mesenchymal transition (EMT), a process where epithelial cancer cells acquire mesenchymal features, is emerging as key determinant of cancer cell invasion and metastasis [2-4]. To metastasise, cancer cells acquire the ability to erode the extracellular matrix, the motility to extravasate into the blood stream, and the plasticity to grow in a different tissue. In all these phases, nutrient supply can be limited and cancer cells experience varying degree of stress [5]. Accordingly, metastatic cells fine-tune their metabolism to adapt to the ever-changing environment [6, 7]. In line with this observation, part of the genetic reprogramming orchestrated by EMT affects the expression of metabolic genes, regulating glucose, lipids, glutamine, and nucleotide metabolism. Yet, to what extent EMT rewires the metabolic network is still unclear. The recent discovery that oncogenic mutations of metabolic enzymes such as fumarate hydratase (FH), succinate

dehydrogenase (SDH) and isocitrate dehydrogenase (IDH) drive EMT [8-10] indicates that the connection between EMT and metabolism is deeper than anticipated. Indeed, these works revealed that components of the metabolic network can directly affect chromatin structure and function, impinging on signalling cascades required for the full activation of EMT [11, 12]. In this review, we describe the role of EMT in tumorigenesis, how EMT affects metabolism, and how, in turn, dysregulation of metabolic genes affect the execution of EMT.

The epithelial-to-mesenchymal transition in cancer

In tissues, epithelial cells are organised in compact layers anchored to the basal lamina. During transformation, some of these cells lose their epithelial features and acquire a mesenchymal phenotype through a process defined as epithelial-to-mesenchymal-transition (EMT). This process is characterised by profound transcriptional [13] and epigenetic changes [14, 15] that lead to the loss of cell-to-cell junctions and the acquisition of a motile and migratory phenotype, enabling the invasion of the basal lamina, which eventually may lead to metastasis. At the molecular level, EMT is dictated by a network of transcription factors (EMT-TFs) that directly or indirectly represses one of the key epithelial markers, E-Cadherin [13, 16]. These EMT-TFs belongs to various family of chromatin interacting family of proteins, including Snail (Snai1 and Snai2), bHLH (Twist1 and Twist2), and zinc finger and E-box binding (Zeb1 and Zeb2). Cross-activation of EMT by other oncogenic stimuli and the identification of non-canonical EMT-TFs such as Kruppel-like-factor (KLF8), the homebox proteins gooseoid (GSC) or fork-head protein (FOXO2), contributes to the great complexity of EMT regulation [13, 16]. Moreover, recent evidence has shown that microRNAs are also potent regulators of EMT, affecting the expression of multiple targets of this cascade [17].

The role of the EMT-TFs in invasion and metastasis has been extensively investigated [16]. *In vivo* experiments using a spontaneous squamous cell carcinoma mouse model showed that the expression of the EMT-TF *Twist1* is sufficient to trigger EMT and the subsequent dissemination of cancer cells into the blood stream. Interestingly, the colonisation of target tissues by these metastatic cells is driven by a mesenchymal-to-epithelial transition (MET) and requires suppression of *Twist1* [18]. Other works identified a primary role of the EMT in breast cancer progression. For instance, *Twist1* controls the ability of aggressive breast 4T1 cells to migrate *in vitro* and to metastasise to the lung *in vivo* [19]. The role of *Twist1* in early dissemination and metastasis was also corroborated in human epidermal growth factor receptor 2 (Her2)-positive mammary cancer cells. It was shown that in early lesions in mouse breast, a subpopulation of cells that express high levels of *Twist1*, low levels of E-cadherin, and markers of Wnt signalling activation, invade the adjacent tissue and lead to early dissemination and subsequent metastasis [20]. Moreover, in mouse skin squamous cell carcinoma, *Twist1* is required in both early and late stages of tumour progression in a gene dosage- dependent

manner [21]. Other EMT-TF are directly involved in breast cancer metastasis. For instance, the expression of *Snai1* in a mouse model of breast cancer activates the dissemination of cancer cells and its deletion dramatically impairs the formation of metastasis [22]. The impact of *SNAI1* activation in the malignancy of breast tumours has been further confirmed by the discovery that the discoidin domain receptor 2 (DDR2), a protein expressed in ductal breast carcinomas, drives invasion *in vitro* and metastasis *in vivo* through the nuclear stabilisation of *Snai1*, via phosphorylation mediated by extracellular related kinase 2 (ERK2) [23]. Even though a series of convincing works established the involvement of EMT in metastasis formation, its real importance in tumour evolution is still questioned. For instance, two groups recently showed that the EMT is dispensable for metastasis in a model of pancreatic [24] and breastcancer [25]. These results suggest that the role of EMT in cancer progression is likely tissue-specific and that it might be implicated in other features of cancer. Indeed, it has recently emerged that EMT, via the expression of EMT-TFs, enables stemness in cancer cells [2, 16]. For instance, an orchestrated signal mediated by *SNAI2* and *SOX9* induces a stem state and promotes tumorigenesis in mammary luminal cells [26], while the ectopic expression of *TWIST1* or *SNAI1* results in the expression of stem markers in human immortalised mammary cells [27]. Moreover, ZEB1-mediated suppression of miR200 favours the expression of polycomb repressor protein Bmi1 [28, 29] and Suz12 [30], two regulators of self-renewal and stemness in breast cells. Further work showed that the acquisition of stem-like properties through EMT activation is involved, at least in part, in both chemoresistance [31, 32] and tumour dormancy [31, 33-35]. These two prominent features of cancer therapy may be interlinked. Seminal work using an elegant *in vivo* model to trace EMT lineage during metastasis showed that EMT-positive cells are responsible for recurrence of lung metastasis after chemotherapy with cyclophosphamide, suggesting that chemoresistance, EMT and dormancy may be part of the same pathway [25].

EMT activation induces a metabolic rewiring

Recent findings indicate that mesenchymal cancer cells have different metabolic needs compared their epithelial counterparts, to satisfy the metabolic demands of increased motility and invasion. Yet, how EMT regulates metabolism is still poorly understood. In the effort to corroborate this link, Shaul and colleagues analysed the expression of metabolic genes in high-grade carcinomas expressing mesenchymal markers using publically available data from almost 1000 cancer cell lines. They found that these mesenchymal cells exhibit high expression levels of 44 metabolic genes. These genes were found upregulated also upon induction of EMT by expression of *Twist1* in human mammary epithelial cells. Among these enzymes, Dihydropyrimidine dehydrogenase (DPYD), an enzyme involved in pyrimidine catabolism, was required for EMT, both *in vitro* and *in vivo* [36] (Figure 1). Importantly, exogenous dihydropyrimidines are sufficient to rescue EMT after silencing of DPYD, suggesting that

these metabolites are a limiting factor during the EMT. However, the how they regulate EMT is currently unknown.

Overall, these results suggested that metabolic rewiring is required to complete the reprogramming orchestrated by EMT. In further support of these findings, it was found that *SNAI1* expression represses the glycolytic enzyme fructose-1,6-bisphosphatase 1 (FBP1), favouring glucose uptake and the diversion of glycolytic carbons towards biosynthetic pathways, including the pentose phosphate shunt (Figure 1). Interestingly, FBP1 loss impairs respiration and the activity of respiratory chain complex I [37]. Activation of glycolysis by EMT was also observed in breast and prostate cancer cells, where it is required for both cytoskeleton remodelling and increasing cell traction [38]. Glycolysis is targeted by EMT also in non-small cell lung cancer cells (NSCLC), where ZEB1 activate the expression of glucose transporter 3 (*GLUT3*) [39]. However, the metabolic reprogramming upon EMT in NSCLC is controversial. For instance, the treatment of NSCLC with TGF- β induces a shift from glycolysis to OXPHOS and leads to an overall increase in amino acids, in particular in glutamate, via a higher flux of carbons through the TCA cycle. Mechanistically, this shift from glycolysis to OXPHOS is achieved by a selective repression of pyruvate dehydrogenase kinase 4 during EMT [40]. Finally, EMT induction by TGF- β in colon cancer cells elicits the nuclear translocation of pyruvate kinase M2 (PKM2) and the silencing of PKM2 prevents EMT triggering by TGF- β in these cells [41] (Figure 1).

Other metabolic pathways are targeted during EMT, including lipid metabolism (Figure 1). For example, EMT activation by either TNF α or TGF- β favours the accumulation of unsaturated triacylglycerides in DU145 prostate cancer cells [42]. Furthermore, the activation of EMT by overexpression of *SNAI1* suppresses transcriptional regulators of the lipogenesis carbohydrate-responsive element binding protein (ChREBP) leading to the silencing of both fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) [43]. Finally, another pathway required during EMT is glutaminolysis (Figure 1): lung cancer cells that undergo an EMT become increasingly sensitive to Glutaminase-1 (GLS1) inhibitors [44].

As discussed above, EMT activation is involved in both chemoresistance and tumour dormancy. Even though the role of metabolism in these processes is largely unknown, recent works suggest that metabolic rewiring can be important in both chemoresistance and tumour dormancy. For instance, EMT-positive breast cells that are responsible for recurrent lung metastasis after chemotherapy increased the expression of metabolic enzymes such as drug transporters, aldehyde dehydrogenase (ALDHs), cytochrome P450s, and enzymes of glutathione metabolism [25] (Figure 1). Likely, these metabolic changes protect the cells from oxidative stress experienced during therapy. Furthermore, deletion of *Twist1* or *Snai1* in chemoresistant pancreatic cancer cells increase the expression of a nucleosides transporter, which leads to increase uptake of the anticancer drug gemcitabine [24]. The

link between EMT, metabolic alterations, and tumour dormancy remains mainly indirect. It is widely known that during tumour dormancy, cancer cells undergo proliferative arrest and enter quiescence [34]. Therefore, it not surprising that this change in proliferation rate is accompanied by a metabolic rewiring. For instance, pancreatic ductal cancer cells surviving after oncogene ablation acquire stem-like traits and are dependent on oxidative phosphorylation for survival [45]. In addition, quiescent leukaemia stem cells (LSC) rely on mitochondrial metabolism: targeting the oxidative phosphorylation through BCL-2 inhibition is sufficient to eradicate LSC population [46]. However, the impact of EMT-TFs in regulating these metabolic alterations during dormancy is largely unknown and it might be related to the dynamic shift between EMT and MET that occurs on tumour circulating cells [47]. Overall, these results suggest that metabolic reprogramming is instrumental to the phenotypic shift observed during the EMT. Whether these metabolic changes are simply required to fulfil the energy requirements of more aggressive cells or to support some of the signalling cascades involved in this process is still unknown.

Metabolic reprogramming activates the epithelial- to-mesenchymal transition

Recent evidence suggests that the link between EMT and metabolism is mutual and, in some circumstances, alterations of metabolism can drive EMT. The next part of the review describes how the dysregulation of metabolic pathways is associated with EMT induction. These findings are summarised in Figure 2.

Glycolysis

Aerobic glycolysis is the most distinctive metabolic alteration of cancer cells [1, 48] but the role of glycolytic enzymes in the induction of EMT has emerged only in the last years. Phosphoglucose isomerase (PGI) is a glycolytic enzyme that converts glucose-6P to fructose 6-P. This enzyme was found to be secreted by cancer cells and to act as cytokine, taking the name of autocrine motility factor (AMF). Overexpression of PGI/AMF causes a NF- κ B-dependent stabilisation of ZEB1 and ZEB2 in breast cancer cells [49] and ectopic expression in normal epithelial breast MCF10A triggers EMT [50]. Importantly, suppression of PGI/AMF leads to reverse MET in lung fibrosarcoma [51] and endometrial cancer cells [52]. As described above, the expression of the glycolytic enzyme fructose-1,6-biphosphatase (FBP1) blocks the induction of EMT mediated by SNAI1 in luminal breast cells. The silencing of FBP1 favours EMT also in gastric cells *in vitro* [53]. Other glycolytic enzymes are involved in EMT induction. For instance, the silencing of Aldolase A (ALDOA), an enzyme that converts fructose-1,6-bisphosphate to glyceraldehydes-3-phosphate and hydroxy-acetone, impairs lung squamous carcinoma cell motility and tumorigenesis and this phenomenon is associated with repression of mesenchymal markers [54]. Furthermore, silencing of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibits EMT by repressing *SNAI1* in colon cancer [55]. Finally, overexpression of lactate

dehydrogenase (LDH), the enzyme that converts pyruvate to lactate, leads to increased migration and invasion in bladder cancer cells [56].

Mitochondrial metabolism

Mitochondrial dysfunction is a key feature of cancer and has been frequently associated with increased aggressiveness and metastatic potential [57, 58]. Yet, the mechanistic link between mitochondrial dysfunction and EMT have only recently been investigated. In 2014 it was shown that mitochondrial dysfunction induced by depletion of mitochondrial DNA in breast cells leads to profound morphological and molecular changes that resembles EMT, including increased expression of EMT-TFs, metalloproteases and suppression of E-cadherin, triggered by a Calcineurin A (CaN)-dependent mechanism [59]. In support of this finding, we recently found that the downregulation of mitochondrial genes is a common feature of highly aggressive cancers, and that it significantly correlates with the activation of EMT across 21 different types of cancer [60]. More recently, we and others have demonstrated that EMT is a key signature of tumours harbouring mutations in the Tricarboxylic Acid (TCA) cycle enzymes *FH*, *SDH* and *IDH* [8-10].

Fumarate hydratase is the enzyme that converts fumarate to malate. Mutations of this enzyme lead to Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) [61] and other tumour types, including paragangliomas and pheochromocytomas [62, 63], whilst *FH* deletions have been found in neuroblastoma [64]. *FH*-mutant renal tumours are highly aggressive and metastasise even when small [65]. However, the mechanisms underpinning this aggressiveness are still under investigation. We recently demonstrated that *FH*-deficient cells exhibit a striking mesenchymal phenotype, linked with the expression of an EMT signature [8]. The link between *FH* and EMT was also observed in nasopharyngeal carcinoma, where *FH* is transcriptionally repressed by the lymphoid-specific helicase (LSH) [66]. Mechanistically, we found that fumarate, which accumulates in *FH*-deficient cells and tumours, is responsible for the induction of EMT by inhibiting the TET-dependent demethylation of the anti-metastatic microRNA miR200 [8], known inhibitors of both *SNAI2* [67] and *ZEB1* [68] (Figure 3).

Another TCA cycle enzyme implicated in EMT is Succinate dehydrogenase (*SDH*), a component of the respiratory chain that converts succinate to fumarate. *SDH* mutations have been described in pheochromocytomas and paragangliomas [69-72], sporadic renal cancer [73] and gastrointestinal stromal tumours [74, 75]. A recent study revealed that human metastatic pheochromocytomas and paragangliomas harbouring *SDHB* mutations are invasive and exhibit activation of EMT-TFs such as *SNAI1* and *SNAI2*, suggesting the induction of EMT in these tumours [10]. Consistently, it was shown that loss of *SDHB* in chromaffin cells induces these EMT-TFs and leads to the epigenetic silencing of keratin-19 [76, 77]. Importantly, the migratory phenotype of these cells is reversed by the use of a

DNA methylation inhibitor, decitabine. The link between *SDHB* deficiency and EMT was also shown in colorectal cancer, where the silencing of *SDHB* promotes cell migration and invasion in a TGF- β /SNAIL-mediated-process [78], and also in ovarian cancer [79]. Finally, loss of the assembly factor SDH5 [80], induces EMT in lung cancer cells and metastasis *in-vivo* through activation of a glycogen-synthase kinase (GSK-3 β)- β -catenin axis [81]. Although these studies did not focus on the accumulation of succinate as a mediator of EMT, we recently found that succinate, similarly to fumarate, can induce the epigenetic suppression of miR200 and subsequent EMT induction in *Sdhb*-deficient epithelial kidney cells [8] (Figure 3).

Other TCA cycle enzymes recently appeared in the spotlight of cancer biology and EMT are Isocitrate Dehydrogenases (IDHs), enzymes involved in the oxidative decarboxylation of isocitrate to α -ketoglutarate (α KG). Three isoforms of IDH have been identified: cytosolic IDH1 and mitochondrial IDH2 are NADP⁺-dependent enzymes, while mitochondrial IDH3 is a NAD⁺-dependent protein. Heterozygous mutations in either *IDH1* or *IDH2* have been found in gliomas and leukaemia [82-84]. *IDH1* and *IDH2* mutations are neomorphic and lead to the production of 2-hydroxyglutarate (2HG), which was shown to induce EMT. Similar to what was described for FH and SDH deficient cells, EMT in IDH-mutant cells is driven by alterations of the *miR200-Zeb1* axis (Figure 3). This phenomenon was observed in breast tumours [9], and in colorectal cancer cells [9, 85].

Finally, another TCA cycle enzyme associated with EMT is citrate synthase (CS), the enzyme that catalyses the first committed step of the TCA cycle. Silencing of CS induces morphological and molecular changes in human cervical carcinoma cells that resemble EMT, and promotes metastasis *in vivo*. The molecular mechanisms responsible for this phenotype are not clear, but it is possible that the mitochondrial dysfunction observed in these cells is involved [86]. However, more recent experiments indicate that CS is upregulated in other tumour types such as ovarian cancers and that its silencing impairs both motility and invasion of tumour cells *in vitro* [87]. Therefore, the role of CS in tumour progression is still unclear and it might be tissue-dependent.

Lipid metabolism

Several recent reports support the connection between lipid metabolism and EMT. For instance, the overexpression of acetyl-CoA synthetase (ACSL1 and ACSL4) and steroyl-CoA desaturase (SCD) can activate EMT in colorectal cancer, leading to increased migration, invasion and colony formation *in vitro*. Importantly, the expression of these three enzymes is associated with poor prognosis in stage II colorectal cancer patients [88]. In addition, elevated fatty acid uptake via CD36 activates a Wnt-dependent EMT in hepatocellular carcinoma (HCC) [89]. Of note, in human oral cancer cells CD36-positive cells are responsible for cancer initiation and metastasis *in vivo*. However, in the latter model the EMT is not involved in the formation of metastasis [90]. Other enzymes of lipid metabolism have

been identified as EMT regulators. For instance, silencing of ATP citrate lyase (ACL) reverses EMT in lung cancer and impairs stemness in both lung and breast cells by SNAI1 repression [91]. Moreover, silencing of acetyl-CoA carboxylase 2 (ACC2) reverted the EMT transition triggered by glucose stress, triglyceride deposit and malonyl-CoA accumulation in kidneys [92]. Interestingly, treatment of cancer cells with fatty acids such as arachidonic or linoleic acid elicits an EMT that is downstream of the oncogenic cascades mediated by SRC, NF- κ B and FAK [93, 94].

Glutaminolysis

Most cancer cells depend on glutamine utilisation [48], and the role of glutaminolysis in EMT has been recently investigated. The inhibition of glutaminolysis by targeting GLS1 impairs *in vivo* metastasis through repression of SNAI1 [95]. On the contrary, the expression of GLS2, the mitochondrial isoform of glutaminase, inversely correlates with stage, tumour size, and prognosis in HCC. However, this phenomenon is independent of GLS2 glutaminase activity and involves the GLS2-mediated stabilisation of the EMT-related microRNA miR-34a *via* the Dicer complex [96]. These results suggest that the effects of glutamine catabolism on EMT might be context-dependent and more work is necessary to elucidate the importance of glutaminolysis in this process.

Conclusions and future perspective

EMT is a fundamental biological process involved in development, fibrosis, and wound healing [4]. Recent evidence indicates that this process is also involved in tumour initiation and metastasis. EMT elicits a complex phenotypic switch that endows cancer cells with ability to survive during invasion, dissemination, and metastasis. This flexibility is achieved at least in part by the rewiring of the metabolic network. As discussed above, EMT, via EMT-TFs, orchestrates profound metabolic changes that allow the cell to sustain the energy needs of a cancer cell in an ever-changing tumour micro environment. Yet, the role of metabolism in EMT seems to go beyond these simple enabling features. Indeed, the observation that dysregulation of cellular metabolism, in some circumstances, drives EMT indicates that parts of the metabolic network could act as a core component of the signalling cascade elicited by the EMT (Figure 4). The data discussed in this review corroborate this hypothesis and indicate that specific metabolic alterations could lead to chromatin changes that are required for the activity of EMT-TFs. Several questions arise. For instance, it is still unclear why different sources of mitochondrial dysfunction converge on EMT. In an interesting parallel, EMT induction is associated with bypass of oncogene –induced senescence [97]. Given that senescence is a common outcome of metabolic stress [98] it is possible that induction of EMT could provide cells with the sufficient plasticity to survive and proliferate in the presence of metabolic defects or under nutrient stress. In this scenario, metastasis could be seen as a strategy to explore novel, and more favourable, metabolic niches, and increased motility the means to this goal. Another outstanding question in the field is to

what extent the EMT observed in metabolically-impaired cells contributes to tumorigenesis. The fact that EMT is the most enriched gene signature in FH and SDH-deficient cells seems to support a driving role of EMT in these tumours. It would be important to validate this hypothesis by assessing tumorigenesis in FH- or SDH-deficient models where EMT-TFs are ablated. Finally, the fact that EMT shows unexpected metabolic facets offers interesting therapeutic perspectives (Fig.4). Indeed, EMT could be potentially reverted by targeting specific metabolic enzymes, or targeting the metabolism-dependent epigenetic reprogramming, eventually limiting cancer metastasis. Consistently, inhibitors of mutant IDH were shown to revert glioma cells to a more differentiated state [99], and the DNA methylation inhibitor, decitabine, impairs the invasive phenotype of SDH-deficient cells [77]. Along this strategy, a recent screening was designed to identify small molecules that could revert the mesenchymal phenotype of cancer cells activating E-cadherin transcription. Interestingly, it was found that protein kinase A (PKA) activation by increasing cyclic AMP (cAMP) levels, is sufficient to trigger a mesenchymal-to-epithelial transition (MET) in aggressive breast cancer cells, through activation of the histone demethylases PHF2. cAMP is a key second messenger and its levels are tightly controlled by the energy state of cells [100]. Therefore, it is tempting to speculate that metabolic alterations, through regulation of cAMP levels, are necessary for full EMT activation and that altering metabolism could be a tempting strategy to modify cell phenotype and, more importantly, aggressive features of cancer.

Overall, in this review we provided compelling evidence that EMT and metabolism are intertwined. Understanding the underpinning molecular determinants of this relation is revealing novel insights into how tumours are formed and disseminate, and will potentially provide novel targets for targeting metastasis, the major killer in cancer.

Competing interests

The authors declare no competing interests.

Authors' contribution

MS and CF jointly wrote the manuscript.

Authors' information

MS is a Research Associate in the laboratory of CF. CF is a group leader at the MRC Cancer Unit, University of Cambridge, Cambridge, UK. MS and CF are funded by an MRC Core Funding to the MRC Cancer Unit.

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Figure legends

Fig.1 EMT controls metabolic reprogramming

EMT transcription factors (EMT-TFs) control the expression of metabolic genes of different pathways such as glycolysis, lipid metabolism, and mitochondrial metabolism, and glutaminolysis. Specifically, EMT-TFs suppress the expression of fructose-1,6-bisphosphatase 1 (FBP1), fatty acid synthase (FASN), acetyl-coA carboxylase (ACC), nucleoside transporter, and pyruvate dehydrogenase kinase 4 (PDK4), whilst enhance the expression of dihydropyrimidine dehydrogenase (DPYD), glutaminase 1 (GLS1), enzymes of glutathione metabolism, cytochrome P450, aldehyde dehydrogenases, and glucose transporter 3 (GLUT3). Red dashed arrows indicate the metabolic nodes regulated by EMT-TFs. TCA=tricarboxylic acid cycle.

Fig.2 Metabolic genes control EMT.

Aberrant expression of metabolic enzymes of glycolysis (orange), lipid metabolism (purple), glutaminolysis (blue), mitochondrial metabolism (green), leads to EMT. Red dashed arrows indicate the link between specific metabolic pathway/metabolites and EMT. ACC=acetyl-CoA carboxylase; ACL=ATP citrate lyase; ACSL=acetyl-CoA synthetase; ALDOA=aldolase A; CaN=calcineurin A; Cl-CV=respiratory chain complexes I-V; CoQ=coenzyme Q; CS=citrate synthase; CytC=cytochrome C; FBP1=fructose-1,6-bisphosphatase 1; FH=fumarate hydratase; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; GLS=glutaminase; IDH=isocitrate dehydrogenase; LDHA=lactic dehydrogenase A; PGI=phosphoglucose isomerase; PKM2=pyruvate kinase M2; SCD=steroyl-CoA desaturase; SDH=succinate dehydrogenase.

Fig.3 EMT activation by mutations in *FH*, *SDH* and *IDH* requires epigenetic reprogramming.

Schematic representation of how mitochondrial metabolites accumulated upon mutation of the indicated TCA cycle enzymes activate the EMT. A common pathway affected by these metabolites is the epigenetic suppression of a family of antimetastatic microRNAs, miR200, *via* the inhibition of histone demethylases (KDMs) and DNA demethylases (TETs). Of note, in the case of 2HG, the suppression of miR200 is indirect, and occurs via activation of Zeb1/2. See the text for more details. FH=fumarate hydratase; SDH=succinate dehydrogenase; IDH=isocitrate dehydrogenase.

Fig.4 Integration between oncogenic signalling, metabolic transformation, and epigenetic reprogramming during EMT

EMT requires the coordinated activation of multiple cellular processes, here represented as gears within a clockwork. Each of these components are essential for the full activation of EMT. As consequence, the inhibition of parts of this clockwork hampers the full activation of the EMT. For

724 instance, inhibition of mutant IDH, or activation of PKA can block EMT. PKA=protein kinase A;
725 IDH=isocitrate dehydrogenase.
726